REMARKS

The Office Action mailed February 13, 2003 has been received and carefully considered. Applicants have amended the claims in a manner which they believe addresses the Examiner's concerns, and provide the following comments. Applicants invite the Examiner to contact the undersigned if the Examiner has any questions and/or to expedite allowance. New claims 45-48 find support throughout the application as originally filed, more particularly on page 16, lines 7-34. No new matter is added as a result of the amendments.

35 U.S.C. § 112 ¶ 2

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-44 were rejected under 35 U.S.C. §112 ¶ 2 on page 2 of the Office Action as being indefinite. Applicants appreciate the Examiner's comments and believe that the amendments made to claims 1 and 14 address the Examiner's concerns. As claims 2, 12 and 13 have been cancelled, the rejection is moot regarding those claims. More particularly, claim 1 has been amended to recite "the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed using the same sample or subsample substantially simultaneously" while the text "in the same determination routine" has been deleted from the claim. This recitation is now included in a new claim 45 and the specification explains the meaning of this term, e.g., at page 6, lines 11-13. The Examiner stated that "substantially simultaneously" was ambiguous, however, the application as filed, considered as a whole, provides one skilled in the art the knowledge necessary to define the claimed invention, e.g., see page 16, lines 29-33. Claims 14 and 16 have been amended to depend on claim 4 instead of on claim 6. Claims 21, 22, 25-27, 32 and 39 have been amended to correct spelling errors.

The rejection is respectfully traversed.

35 U.S.C. §102(b)

> A person shall be entitled to a patent unless -
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> (b) the invention was patented or described in a printed publication in this or a foreign country or in a public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Generally, the claimed invention is directed toward a substantially simultaneous

determination of concentration of sperm cells and the proportion of live sperm cells (viability), in a semen sample. The total number of sperm cells and the proportion of live sperm cells are important factors for achieving optimal fertility. It has been found that there seems to be a cut-off value below which the fertility drops below a certain percentage thus being below the profitable fertility rates. The total number of sperm cells and the proportion of live sperm cells required for obtaining this minimum acceptable fertility is different for different species. Furthermore, the total number of sperm cells and the proportion of live sperm cells differ between males of the same species and between different ejaculates for the same male, thus leading to a cut-off value differing between males of same species and between different ejaculates for the same male. Accordingly, the claimed invention provides a method for performing a determination for each ejaculate to obtain a maximum number of insemination doses from each ejaculate.

The precision with which the total concentration of sperm cells and the proportion of live sperm cells is determined is of great importance, since a precise determination of these values provides: (a) the possibility of evaluating single ejaculates on the basis of a single determination, and (b) the possibility of using ejaculates having values closer to any predetermined cut-off value, since a more precise determination means that the safety distance to any predetermined cut-off value may be chosen according to the precision with which the determination is performed.

The substantially simultaneous determination of the total concentration of sperm cells and the proportion of live sperm cells from a single sample increases the precision of the measurement in that the possibilities of mistakes and errors during handling and pipetting are reduced. In the claimed invention only one sample is prepared for determination whereas prior art methods use more determination routines and more measurements on samples prepared differently to obtain the total concentration and the proportion of live cells. As a result, the sources of errors when handling and pipetting the samples and when measuring the number of samples are increased and the variation on the resulting determinations are thus equally increased. Therefore, when the methods used for determination of the total sperm concentration are separate from the determination of the proportion of live sperm cells, the variation will necessarily be larger than by combining the two determinations in a single measurement. However, for some purposes it may be useful or desirable to make more determinations on the same ejaculate to exclude possible determination failures. Generally, however, a single

determination should suffice.

Claim 1 and dependent claims 2-5, 9, 13, 16-19, 31, 32, 33, 36, 39, 41 and 43 under 35 U.S.C. 102(b) were rejected as being anticipated by GB 2 214 518. "Anticipation under 35 U.S.C. § 102 requires the disclosure in a single piece of prior art of each and every limitation of a claimed invention." (*Electro Med. Sys. S.A. v. Cooper Life Sciences*, 32 U.S.P.Q.2d 1017, 1019 (Fed. Cir. 1994)). As discussed above, the claimed invention is directed to a method for the determination of the total concentration of sperm cells in a semen sample and the proportion of live sperm cells therein, comprising subjecting the semen sample or a diluted subsample of the semen sample to selective staining and determining the total concentration of the sperm cells and the proportion of live sperm cells by means of a detection means responsive to the selective staining, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed substantially simultaneously.

However, GB 2 214 518 discloses a method wherein measurement of the fluorescent intensity F_x of different samples provides a measure of the cell concentration and the percentage of living cells in the sperm samples. The method comprises dissolving Propidium Iodide in a buffer and subsequently measuring the fluorescent intensity, adding the sperm sample and measuring the intensity, adding a membrane-permeabilizing agent to the cell sample and measuring the intensity, adding a buffer and a permeabilizing agent to the dyestuff 1 solution and measuring the intensity, measuring the intensity of the pure buffer, adding pre-diluted sperm mixture to the buffer and measuring the intensity, whereafter the cell count and the ratio of intact cells may be calculated. The process of GB 2 214 518 thus comprises measuring the emission intensity of six samples prepared in different ways so as to obtain a set of values from which the cell count and the ratio of intact cells may be determined. Thus, the result is obtained by a complex procedure involving many routines and different steps of addition of agents, and several different sperm samples, and measuring of intensities for each step.

In contrast, Applicants' invention utilizes a single sample or diluted subsample to determine the necessary sperm concentrations without further manipulation of the sample. Applicants have found a way to reduce the number of steps required and make an accurate determination which is not disclosed in GB 2 214 518.

Applicants respectfully submit that claim 1 and those which depend therefrom are not anticipated by GB 2 214 518. Applicants respectfully traverse the rejection.

35 U.S.C. §103(a)

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-11, 13-29, 31-33, 36-39, 41, 43, and 44 were rejected under 35 U.S.C. § 103(a) as being obvious over US 4,559,309 or JP 8-332098 or Live/Dead Viability Kit or Garner et al. or GB 2 214 518 in combination with EP 586 183 or WO 93/16385. Claim 30 was rejected over the same art as was applied to claims 1-11, 13-29, 31-33, 36-39, 41, 43, and 44 in further combination with Clay *et al.* Claims 32-34, 39, 41 and 42 were rejected over the same art as applied to claims 1-11, 13-29, 31-33, 36-39, 41, 43, and 44 in further view of Sexton or Januskauskas *et al.* or Belorkar *et al.* or Bostofte *et al.*

Initially, Applicants respectfully point out that there is no motivation found in any of these references to combine them. Furthermore, as stated above, GB 2 214 518 discloses a method wherein the fluorescence is measured on a number of different samples, each being prepared in a different way. Thus, even if, arguendo, GB 2 214 518 could be properly combined with EP 586 183 or WO 93/16385 to produce an artificial disclosure comprising the use of a flow cytometer, several measurements would still be required using different samples or the same sample modified in different ways. GB 2 214 518 or in EP 586 183 or WO 93/16385 do not disclose how to reduce the number of steps and/or measurements to determine the total concentration of sperm cells and proportion of live sperm cells substantially simultaneously from a single sample or subsample.

The Examiner further stated that independent claims 1, 32 and 39 are unpatentable in view of US 4,559,309, JP 8-332098, Live/Dead Viability Kit or Garner et al. in combination with EP 586 183 or WO 93/16385. Applicants submit that the combination of these references is improper because they do not provide any motivation to do so. Even the improper combination would have failed to render obvious Applicants' derived invention. These references fail to disclose the advantage of simultaneous determination of total concentration and proportion of live sperm cells, *i.e.* the more precise result which is obtainable using the method of the claimed invention or the usefulness of this more precise result, as further explained below. The improper

combination of references also fails to suggest the invention defined in Applicants' claims, as also discussed below.

Juonala has found a correlation between motility and litter size, and it has further been shown that there is a correlation between the total concentration of sperm cells and the proportion of live sperm cells, and the litter size.

For the production of semen doses, it is of great importance to be able to select the ejaculates carefully so that for example ejaculates potentially providing a too small litter size may be rejected before insemination so as to increase the overall mean litter size produced. The claimed invention provides a more precise result due to the substantially simultaneous determination of total concentration and the proportion of live sperm cells in each ejaculate and thus allows for rejection/acceptance of single ejaculates.

Furthermore, even if US 4,559,309, were combined with either EP 586 183 or WO 93/16385, the method of the invention would not be achieved. In US 4,559,309, the sample is stained with Rhodamine 123, staining the mitochondria in the tail and the middle part of the sperm cell, while the other stain, ethidium bromide, stains the head of the sperm cell. Thus, different parts of the sperm cell are stained by the different stains, and since a semen sample may comprise up to 30 % of loose tails or heads of sperm cells depending on the quality of the semen sample, the total concentration of sperm cells can not be very accurately determined, but is likely to be overestimated in cases where many loose heads or tails are present. Thus, especially when the semen quality is low, the concentrations are significantly overestimated. Another suggested approach is to stain all sperm cells with Acridin Orange. However, a pre-treatment of the sample is necessary before staining (cf. col. 5, lines 25-28). Furthermore, Acridin Orange breaks the membrane so that all sperm cells are killed whereby only the total concentration can be determined and not the proportion of live sperm cells so that no information about viability can be obtained.

In JP 8-332098, the esterase activity is labeled by staining the entire sperm cell and also the mitochondria in the tail and the middle part of the sperm cell, so that the concentration tends to be overestimated when the semen comprises many loose heads, tails and middle parts, as also mentioned above.

The improper combination of any of these references with the method suggested in EP 586 183 or WO 93/16385, would still result in an overestimated total concentration and/or an

overestimated proportion of live sperm cells, since there is no suggestion in none of the references as how to overcome these difficulties, since even the problem of overestimation has not been mentioned. The improper combination also would not have suggested the claimed invention because none of these references suggests (or discloses) the substantially simultaneous determination of the total concentration of sperm cells and the proportion of live sperm cells from a simple sample or subsample.

The Examiner further refers to Live/Dead Sperm Viability Kit and Garner et al., both using SYBR-14 and PI for staining. However, neither Garner nor Live/Dead Sperm Viability Kit proposes to determine the concentration and the proportion of live sperm cells substantially simultaneously by any method, much less the method discovered by Applicants, and defined in the claims.

The methods suggested in both EP 586 183 and WO 93/16385 for measuring the concentration in a sample are both developed for measuring blood samples and even if it was proper to combine them with Live/Dead Sperm Viability Kit and/or Garner et al., there is no indication that the sensitive live sperm cells would be able to survive the determination procedure and provide a correct measure of the proportion of live sperm cells. Rather when combining the Live/Dead Sperm Viability Kit or Garner et al. with EP 586 183 and/or WO 93/16385 one would obtain two different determinations; one for obtaining the total concentration of sperm cells and one for obtaining the proportion of live sperm cells.

The references fail to provide any indication of the advantages obtainable by combining the measurements in a single determination. For at least this reason, there is no motivation for a skilled person to combine the measurements. The precise determination of total concentration and number of living sperm cells obtainable according to the claimed invention makes it possible to approach closer the cut-off value for individual species / males / ejaculates and thereby produce a higher number of insemination doses.

By using the methods of the claimed invention to determine total sperm concentration and proportion of live sperm cells and evaluate the semen quality on the basis of these determinations, a significant increase in insemination doses may be obtained. For example for boars, the increase in insemination doses may be up to 40 % compared to traditional methods. These unexpected results of the simultaneous determination of total concentration and proportion of live sperm cells are not disclosed in any of the references cited by the Examiner. The fact that

each individual ejaculate may be evaluated on the basis of a single measurement instead of having to use the mean value of several measurements on the same or a number of ejaculates provides the unexpected possibility of selection of valuable ejaculates for insemination and maximizing the number of semen doses that can be produced from an ejaculate.

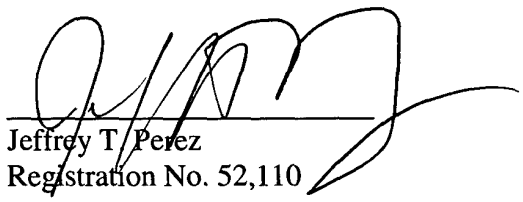
Prior art references in combination do not make an invention obvious unless something in the prior references would suggest the advantage to be derived from combining their teachings. *In re Sernaker*, 217 U.S.P.Q. 1, 6 (Fed. Cir. 1983). A combination may be patentable whether it be composed of elements all new, partly new or all old. *Rosemont, Inc. v. Beckman Instruments, Inc.*, 221 U.S.P.Q. 1, 7 (Fed. Cir. 1984). There must be something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination. *Lindemann v. Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 221 U.S.P.Q. 481, 488 (Fed. Cir. 1984). *Interconnect Planning Corporation v. Feil, et al.*, 227 U.S.P.Q. 543, 551 (Fed. Cir. 1985). In the present case there is no such motivation. This is particularly true given that none of the references cited by the Examiner recognized that by a simultaneous determination of the total sperm concentration and the number of living sperm cells, the variation may be reduced so that a higher precision is obtained. The rejection is respectfully traversed.

CONCLUSION

Applicants assert that the above-referenced application is in condition for allowance. Reconsideration and allowance of all pending claims is respectfully requested. Should any outstanding issues remain, the Examiner is invited to telephone the undersigned at 202-955-1500.

Respectfully submitted,

HUNTON & WILLIAMS



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